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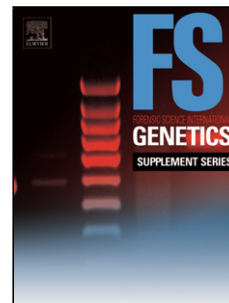
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Authors: James Gooch, Chang Rong Chua, Vincenzo Abbate,
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Fluorogenic Substrates for the Detection of Saliva

James Gooch^a, Chang Rong Chua^b, Vincenzo Abbate^a, Nunzianda Frascione^{a,*}

^aKing's Forensics, Analytical & Environmental Sciences Division, King's College London, 150 Stamford Street, London, SE1 9NH, UK.

^bDepartment of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543, Singapore.

* Corresponding author. Tel.: +4420-7848-4462

E-mail address: nunzianda.frascione@kcl.ac.uk

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ABSTRACT

The potential of fluorogenic substrates to detect and identify human saliva *in situ* was first explored using commercially available substrate Boc-VPR-AMC. The substrate was applied to a range of saliva dilutions deposited on glass microscope slides. A positive fluorescence response was observed immediately after application against each deposit up to a 1:8 dilution. In an attempt to improve assay sensitivity, a novel substrate Ac-VPR-Rho110-Ac, utilising a Rhodamine-110 fluorophore, was prepared using a solid-phase peptide synthesis protocol previously reported by our research group. Application of Ac-VPR-Rho110-Ac to saliva deposits demonstrated a successful increase in assay sensitivity limits, allowing stains of up to 1 in 128 dilution to be detected.

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1. Introduction

The Office for National Statistics estimates that in 2016/2017 alone, over 5 million criminal offences occurred within the UK [1]. Of these offences, almost 50% remain unsolved with no recorded suspect [2]. As the vast majority of criminal identifications made by forensic means involve the use of DNA, there is continuing pressure to develop rapid and sensitive techniques capable of detecting and identifying genetic material deposited as biological fluid traces during a criminal act.

At present, the most widely used methods to identify deposits of saliva include the Phadebas® test, the RSID™-Saliva test and the SALigAE® test, all of which exploit reactions with the single most abundant salivary protein, α -amylase [3]. Although these tests have proven effective within a casework scenario, they often suffer from caveats of low specificity [4] and lack of ability to localise fluids and attribute their tissue type at the same time.

Our research group has recently demonstrated the use of fluorogenic peptide substrates as successful assays for the simultaneous detection and identification of body fluid targets *in situ* [5]. These substrates utilise fluorophores that are quenched as a result of conjugation to an amino acid chain. Incubation of the substrate with a body fluid-specific protease results in the separation of the peptide-fluorophore bond and emission of fluorescence.

These assays were shown to possess both high target specificity and sensitivity, whilst a ‘turn-on’ fluorescence signal allowed for the visualisation of discrete fluid staining areas. Furthermore, this fluorescence approach was shown to be highly compatible with current DNA profiling processes.

In the course of testing a number of commercially available fluorogenic substrates against different biological fluids in solution, it was observed that substrate Boc-VPR-AMC exhibited a strong positive reaction in the presence of saliva. This substrate utilises a 7-amino-4-methylcoumarin (AMC) fluorophore, which is liberated upon target interaction to produce a fluorescence emission at 440 nm (Fig. 1).

This study therefore documents the use of Boc-VPR-AMC, to visualise *in situ* deposits of saliva within a forensic context, as well as the synthesis of a novel substrate (Ac-VPR-Rho110-Ac) based on the same reactive amino acid sequence, in an attempt to increase testing sensitivity.

2. Materials and Methods

2.1. Materials

Saliva was collected upon informed consent from three donors (KCL Ethics BDM/13/14-26). Substrate Boc-VPR-AMC was purchased from R&D Systems (Abingdon, UK) and diluted to a working concentration of 100 μ M. All amino acids and coupling reagents were purchased from Sigma-Aldrich Ltd (Dorset, UK), while Rhodamine-110 (Rho110) was purchased from Fisher Scientific Ltd (Loughborough, UK).

2.2. Ac-VPR-Rho110-Ac Synthesis

Substrate Ac-VPR-Rho110-Ac was prepared using a solid-phase peptide synthesis protocol previously reported [6]. Electrospray Ionisation Mass Spectrometry (ESI-MS) on a Waters/Micromass (Manchester, UK) ZQ mass spectrometer was used to confirm the identity of the product at each key step of the synthesis (data not shown).

2.3. Microscopy

Saliva dilutions (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128) were applied in duplicate to glass slides in 5 μ l deposits and allowed to dry. Working concentration Boc-VPR-AMC (100 μ M) or Ac-VPR-Rho110-Ac (500 μ M) was pipetted in 5 μ l volumes onto the dried saliva. Fluorescence response of the substrates was recorded immediately after application by an Olympus SZX12 fluorescence microscope using UV (Ex 330-385/Em 420 nm) or GFP (Ex 460-490/Em 510 nm) filters for the commercial and synthesized substrates respectively. Negative reagent-only and saliva-only controls were also utilised and recorded in the same manner.

3. Results and discussion

Immediate ‘turn-on’ signal responses of substrate Boc-VPR-AMC were observed against all saliva deposits up to a 1:8 dilution (Fig. 2a). Stains beyond this limit could not be visualised successfully. The use of negative controls also demonstrated the inherent lack of fluorescence emission of both saliva deposits and unreacted substrate.

Our research group recently reported the development of a solid-phase peptide synthesis protocol for the construction of fluorogenic substrates using the fluorophore Rhodamine-110 [6]. Such substrates are likely to be more sensitive than those using coumarin dyes due to Rhodamine-110’s superior spectral characteristics. Attempts were therefore made to improve

assay performance through the construction of a novel substrate Ac-VPR-Rho110-Ac. After confirming the success of substrate synthesis by mass spectrometry, Ac-VPR-Rho110-Ac was applied to saliva deposits in the same manner as commercial substrate testing. Successful visualization of all *in situ* saliva samples up to a 1:128 dilution, demonstrated a significant increase in assay detection limits (Fig. 2b).

During the application of Ac-VPR-Rho110-Ac, it was noted that the substrate solution exhibited some background fluorescence. However, this was successfully corrected for through changes to microscope parameters. This background signal is likely to originate from dye molecules that were not completed conjugated during synthesis. Further purification to remove any free Rhodamine-110 from the substrate product should therefore be undertaken to prevent potential limitations to assay sensitivity.

4. Conclusion

This proof-of-concept study has demonstrated the potential of fluorogenic substrates as a rapid, portable and simple method for the visualisation of saliva. Commercially available Boc-VPR-AMC was first utilised to detect deposited saliva *in situ* before assay sensitivity was increased through the construction of synthetic substrate Ac-VPR-Rho110-Ac. With substrates now designed by our group for the detection of both semen and saliva, future work is likely to focus on the development of a multiplex substrate system for the detection of multiple fluid types simultaneously.

Funding Source

King's College London.

Conflict of interest

None.

REFERENCES

- [1] Crime in England and Wales: year ending March 2017, Office for National Statistics, 2017.
- [2] Crime Outcomes in England and Wales 2016/17, Home Office, 2017.
- [3] K. Virkler, I.K. Lednev, Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene, *Forensic Sci. Int.* 188(1) (2009) 1-17.
- [4] B.C.M. Pang, B.K.K. Cheung, Applicability of Two Commercially Available Kits for Forensic Identification of Saliva Stains, *J. Forensic Sci.* 53(5) (2008) 1117-1122.
- [5] J. Gooch, B. Daniel, N. Frascione, Application of fluorescent substrates to the in situ detection of prostate specific antigen, *Talanta* 125 (2014) 210-214.
- [6] J. Gooch, V. Abbate, B. Daniel, N. Frascione, Solid-phase synthesis of Rhodamine-110 fluorogenic substrates and their application in forensic analysis, *Analyst* 141(8) (2016) 2392-2395.

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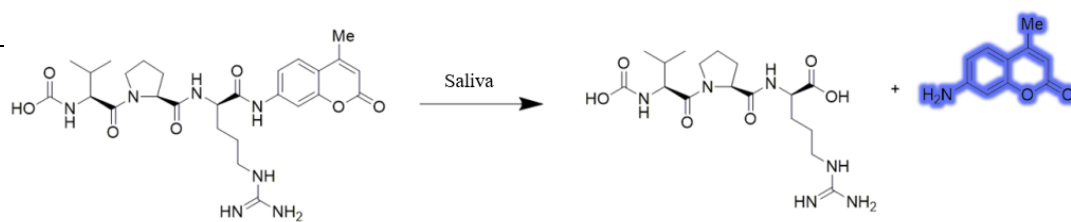


Fig. 1 - Boc-VPR-AMC - A short peptide chain (VPR) terminally labelled with a fluorophore, creating a non-fluorescent substrate system. Resulting protease activity relieves quenching to release highly fluorescent 7-amino-4-methylcoumarin.

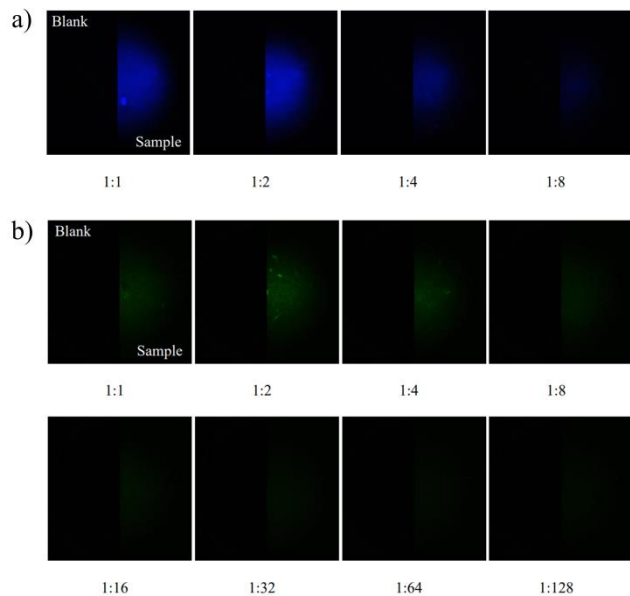


Fig. 2 - Fluorescence response of (a) Boc-VPR-AMC and (b) Ac-VPR-Rho110-Ac to saliva dilutions deposited on glass slides. Negative saliva-only controls are provided on the left side of each image.